

Appl. No. 09/882,193
Response dated November 19, 2003
Reply to Bona Fide Attempt Letter of October 24, 2003

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Listing of Claims:

Claim 1. (Previously presented) A method of counting a single molecule of a target nucleic acid in a sample, said method comprising:

- (i) detecting an optical characteristic of a first quantum dot and a second quantum dot attached to said single molecule of said target nucleic acid, wherein said first quantum dot and said second quantum dot are distinguishable; and
- (ii) resolving said optical characteristic of said first quantum dot and said second quantum dot attached to said single molecule of said target nucleic acid from an optical characteristic of a quantum dot not attached to said single molecule of said target nucleic acid, thereby counting said single molecule of said target nucleic acid.

Claim 2. (Previously presented) The method as in claim 1, further comprising quantitating the target nucleic acid by analyzing the detected optical characteristic.

Claim 3. (Previously presented) The method as in claim 1, further comprising transcribing the target nucleic acid.

Claim 4. (Previously presented) The method as in claim 3, wherein the target nucleic acid comprises DNA and transcribing comprises using a primer which anneals to a conserved region of the DNA and transcribes a polymorphic region of the DNA when extended.

Claim 5. (Previously presented) The method as in claim 4, wherein the primer is biotinylated and the transcribing step produces biotinylated DNA.

Claim 6. (Previously presented) The method as in claim 3, further comprising binding the transcribed target nucleic acid to a substrate.

Claim 7. (Original) The method as in claim 6, wherein the substrate comprises a streptavidin coated surface, support, plate or slide.

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Claim 8. (Previously presented) The method as in claim 6, further comprising removing unbound portions of the target nucleic acid.

Claim 9. (Previously presented) The method as in claim 6, further comprising probing the bound target nucleic acid using a sequence-tagged hybridization probe.

Claim 10. (Original) The method as in claim 9, wherein the target comprises DNA having at least one point mutation and the probing comprises binding the probe to said at least one point mutation of the DNA.

Claim 11. (Original) The method as in claim 9, wherein the target comprises wild type DNA and the probing comprises binding the probe to the wild type DNA.

Claim 12. (Original) The method as in claim 9, further comprising removing non-specifically bound probe.

Claim 13. (Original) The method as in claim 9, wherein each quantum dot has an attached oligonucleotide tag and labeling comprises binding each tag with a complementary sequence of each sequence-tagged hybridization probe.

Claim 14. (Original) The method as in claim 13, further comprising removing unbound quantum dots.

Claim 15. (Previously presented) The method as in claim 13, wherein detecting comprises scanning the substrate with resolution capable of detecting an optical characteristic of a single quantum dot.

Claim 16. (Previously presented) The method as in claim 15, further comprising quantitating the target nucleic acid by analyzing the detected optical characteristic, wherein analyzing comprises counting the number of quantum dots within an area of scanned substrate.

Claim 17. (Previously presented) A method of counting a single molecule of a target nucleic acid in a sample, said method comprising:

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- (i) detecting an optical characteristic of a first quantum dot and a second quantum dot attached to said single molecule of said target nucleic acid, wherein said first quantum dot and said second quantum dot are distinguishable;
 - (ii) resolving said optical characteristic of said first quantum dot and said second quantum dot attached to said single molecule of said target nucleic acid from an optical characteristic of a quantum dot not attached to said single molecule of said target nucleic acid, and
 - (iii) quantitating the target nucleic acid by analyzing the detected emitted fluorescence,
- thereby counting said single molecule of said target nucleic acid.

Claim 18. (Previously presented) A method of counting a single molecule of a target nucleic acid in a sample, said method comprising:

- (i) transcribing said single molecule of said target nucleic acid using a primer comprising an immobilizable label to form an immobilizable target nucleic acid;
- (ii) immobilizing said immobilizable target nucleic acid on a solid support to form an immobilized target nucleic acid;
- (iii) contacting said immobilized target nucleic acid with a sequence-tagged hybridization probe comprising a sequence complementary to a portion of said target nucleic acid;
- (iv) detecting an optical characteristic of a quantum dot conjugate comprising a first quantum dot, a second quantum dot, and a nucleic acid sequence complementary to a portion of said sequence-tagged hybridization probe, wherein said first quantum dot and said second quantum dot are distinguishable;
- (v) resolving said optical characteristic of said quantum dot conjugate from an optical characteristic of a quantum dot conjugate not attached to said immobilized target nucleic acid, thereby counting said single molecule of said target nucleic acid.

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Claim 19. (Previously presented) The method as in claim 1, wherein said optical characteristic is detected by coincidence detection.

Claim 20. (Cancelled)

Claim 21. (Cancelled)

Claim 22. (Cancelled)

Claim 23. (Previously presented) The method as in claim 1, wherein said first quantum dot and said second quantum dot are distinguishable by an optical characteristic which is a member selected from the group consisting of fluorescence spectrum, fluorescence emission, fluorescence excitation spectrum, ultraviolet light absorbance, visible light absorbance, fluorescence quantum yield, fluorescence lifetime, light scattering and combinations thereof.

Claim 24. (Previously presented) The method as in claim 1, wherein said optical characteristic is fluorescence.

Claim 25. (Previously presented) The method as in claim 1, wherein said first quantum dot and said second quantum dot are visually distinguishable as a first color and a second color, respectively.

Claim 26. (Previously presented) The method as in claim 25, wherein said first color and said second color combine to form a third color that is visually or electronically distinguishable from both said first color and said second color.

Claim 27. (Previously presented) A method of selecting a mutant DNA away from a wild type DNA, said method comprising:
contacting mutant DNA attached to a first and a second sequence-tagged hybridization probe with a first and a second oligonucleotide tag comprising a sequence complementary to said first and second sequence-tagged hybridization probes and conjugated to a first quantum dot and a second quantum dot, wherein said first quantum dot and said second quantum dot are distinguishable;

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contacting wild type DNA attached to a third and a fourth sequence-tagged hybridization probe with a third and fourth oligonucleotide tag comprising a sequence complementary to said third and fourth sequence-tagged hybridization probes and conjugated to a third quantum dot and a fourth quantum dot, wherein said third quantum dot and said fourth quantum dot are distinguishable; and

detecting an optical characteristic of the quantum dots, whereby detection of said optical characteristic of said first quantum dot and said second quantum dot detects the mutant DNA and detection of said optical characteristic of said third quantum dot and said fourth quantum dot detects wild type DNA.

Claim 28. (Previously presented) The method as in claim 27, wherein said first quantum dot and said second quantum dot are distinguishable by an optical characteristic which is a member selected from the group consisting of fluorescence spectrum, fluorescence emission, fluorescence excitation spectrum, ultraviolet light absorbance, visible light absorbance, fluorescence quantum yield, fluorescence lifetime, light scattering and combinations thereof, and

wherein said third quantum dot and said fourth quantum dot are distinguishable by an optical characteristic which is a member selected from the group consisting of fluorescence spectrum, fluorescence emission, fluorescence excitation spectrum, ultraviolet light absorbance, visible light absorbance, fluorescence quantum yield, fluorescence lifetime, light scattering and combinations thereof.

Claim 29. (Previously presented) The method as in claim 27, wherein said first quantum dot and said second quantum dot are distinguishable by an optical characteristic which is fluorescence; and

wherein said third quantum dot and said fourth quantum dot are distinguishable by an optical characteristic which is fluorescence.

Claim 30. (Previously presented) The method according to claim 27, wherein said first quantum dot, said second quantum dot, said third quantum dot, and said fourth quantum dot are visually or electronically distinguishable as a first color, a second color, a third color, and a fourth color respectively.

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Claim 31. (Previously presented) The method according to claim 30, wherein said first color and said second color combine to form a visually or electronically distinguishable color different from both said first color and said second color, and

wherein said third color and said fourth color combine to form a visually or electronically distinguishable color different from both said third color and said fourth color.